14 Sorptive Interactions between Soil Particles and Microorganisms

K. C. MARSHALL

Department of Agricultural Science
University of Tasmania
Hobart, Tasmania

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14-1. INTRODUCTION

Studies in soil biochemistry demand an appreciation of direct microbial participation in chemical transformations, as well as of the effects on soil processes of enzymes and metabolic products of microbial origin. Soil microbiologists have long been concerned with the isolation and identification of the predominant microorganisms in soils and with the characterization of the vast range of biochemical reactions catalyzed by these organisms. There has been a distinct shift in emphasis in recent years toward investigations of

the ecology of microorganisms in natural soils (*1–3*). Despite this trend, few investigators have seriously considered the implications of the physicochemical effects of solid–liquid interfaces on microbial behavior in soils.

The presence in soils of a variety of solid particles offers opportunities for sorption processes involving microorganisms to take place. Available evidence suggests that most microorganisms in soil exist in a sorbed state. It is the purpose of this review to consider the evidence for such sorptive interactions, the possible physicochemical mechanisms involved, and the influence of the solid phase of soils on the behavior of microorganisms in soils.

Microbial activity in different soils is often considered in terms of the general physical characteristics of the soils (i.e., per cent sand, silt, clay, organic matter). It becomes obvious in the following discussion that, as natural habitats for microbial growth and metabolism, soils must be evaluated on the basis of both the properties and the organization of their solid constituents. Since the degree of sorption between microorganisms and soil particles is broadly related to the surface area and surface charge properties of the particles, the colloidal fraction (clays and organic matter) normally is the most important soil component with respect to sorptive interactions. With clays, for instance, such factors as type of clay mineral, nature of saturating cations, and organization of clay minerals within the soil matrix may be more important in terms of comparative microbial activity in different soils than the absolute amounts of clay present. Oriented clay skins or clay cutans (*4*) formed around large, relatively inert soil particles such as quartz are an excellent example of the type of organization in soils that must be important in sorptive interactions involving microorganisms.

Throughout this chapter, the names of bacteria used by various authors have been altered to conform with those given in *Bergey's Manual of Determinative Bacteriology* (*5*).

14-2. METHODS OF STUDYING SORPTIVE INTERACTIONS

A brief survey of some methods employed in studies on sorption between soil particles and microorganisms is presented below. Although all the methods are open to criticism, the results obtained have provided valuable information on the interactions between microorganisms and the solid phase of the soil.

A. Counting Procedures

Determinations of the quantitative ratio of sorption of bacterial cells to soil particles by early Russian workers were based on the method of Dianowa and Woroschilowa (*6*). The test organism in meat extract broth was added to a

standard amount of soil; the mixture was shaken in sterile water for 1 minute and allowed to stand for 10 minutes. Plate counts were made on the supernatant liquid, and the percentage of cells sorbed calculated by comparison with a control treatment without soil. Karpinskaya (*7*) suggested that insignificant numbers of cells were carried down mechanically with the soil, but it seems that errors would result from this source and from similarities in the sedimentation rates of cells and certain soil particles. The method is also open to criticism in that materials in the culture medium may sorb onto the surfaces of soil particles or cells, thereby altering the charge relationships between these surfaces. A similar method was employed by Peele (*8*), except that he used cultures suspended in sterile water and made a correction for the number of bacteria present in suspensions of uninoculated, unsterilized soil. The percentage sorption was calculated from the formula:

$$\text{per cent sorption} = \frac{A - (B - C) \times 100}{A}$$

where A is the count of untreated bacterial suspension, B is the count of bacterial suspension plus soil, and C is the count of uninoculated soil suspension.

Zvyagintsev (*9*) and Hattori (*10*) have emphasized the need for aggregate disruption in order to desorb cells and hence estimate accurately the numbers of sorbed cells in soils.

B. Microscopy

1. Light Microscopy

The high degree of selective sorption of certain microorganisms on glass surfaces (*11*) limits the usefulness of the Rossi–Cholodny buried slide technique in studies of interactions between microorganisms and soil particles. Direct microscopic observation of aggregate formation in suspensions of bacteria and soils has been used by Karpinskaya (*7*) and Peele (*8*) as a measure of sorption of bacteria by soil particles. The examination of thin sections of soils (*12–14*) promises to provide an excellent means for studying the geometric relationship between microorganisms and the solid particles of natural soils. Fluorescence microscopy also is a valuable tool (*15*), especially for the observation of freshly cut surfaces stained by acridine orange and illuminated by reflected UV light (*16–18*). The use of fluorescent-labeled antibodies to detect specific microorganisms in natural soils (*19–22*) should be a valuable technique for direct studies on sorptive interactions. Disadvantages of these fluorescence techniques are the alteration of surface charge properties of soil particles and cells by the fluorochrome stains and antibodies used, and the possible interference by clays with the fluorescent image observed under the

microscope (*23*). Bohlool and Schmidt (*24*) have recently reported the successful use of gelatin-rhodamine conjugates to avoid nonspecific staining of soil particles when using fluorescein-labeled antibodies to study *Rhizobium japonicum* in soils. Direct observation of the sorption of various microorganisms to ion-exchange resins (*25*) may be useful in the elucidation of the mechanism of sorption of cells to charged soil colloids.

2. Electron Microscopy

Few studies have been made of microorganisms in soils by means of electron microscopy. Nikitin (*26*) has described some remarkable forms of apparent microorganisms in soils but has not presented any evidence for the sorption of bacteria by soil particles, or vice versa. Electron micrographs showing aggregates of bacteria and soil colloids, and the possible sorption of fine clay particles on bacterial cells, have been published by Jackson et al. (*27*). Marshall (*23*) has presented further electron microscope evidence for the sorption of colloidal montmorillonite and illite particles on bacterial cells and has stressed the need to avoid artifacts resulting from surface tension effects during drying of the samples on grids. Shadowing with gold-palladium alloy was found to enhance the outline of the clay particles. The recent introduction of the scanning electron microscope has made it possible to observe the orientation of microorganisms sorbed on the surface of large soil particles (*28,28a*). Although not as convenient as the scanning electron microscope technique, the use of carbon replica techniques with conventional electron microscopy may provide similar information.

C. Electrophoretic Techniques

Electrophoretic methods have been employed to obtain information on the surface charge properties of clays and microorganisms and to study the interactions between the two (*8,23,29–35*). In order to minimize drift and electrode polarization, timings of particles or bacteria in a microelectrophoresis cell are made in both directions and the average velocity calculated (*32*). The electrophoretic mobility may be calculated as follows:

$$\bar{v} = v/X$$

where $\bar{v}$ is the electrophoretic mobility (μ/sec/V/cm), v the velocity of the cells or particles (μ/sec), and X the field strength (V/cm). Further,

$$X = i/K_s q$$

where i is the current (A), K_s the specific conductivity of the buffer solution, and q the cross-sectional area of the cell (cm^2).

The potential at the plane of shear or zeta potential (ζ) can be calculated

from the electrophoretic mobility by use of the Helmholtz–Smoluchowski equation:

$$\zeta = (4\pi\eta\bar{v})/D$$

where η and D are the viscosity and dielectric constant, respectively, in the diffuse double layer. At the low ionic strengths usually employed, the values of η and D are taken as those for water.

The surface charge density (σ) can be determined by substituting the values of ζ and electrolyte concentration in the following equation (*36*):

$$\sigma = (NDkT/2000\pi)^{1/2}(\sum c_i(\exp^{-Z_i\varepsilon\zeta/kT} - 1) + \sum c_j(\exp^{+Z_j\varepsilon\zeta/kT} - 1)^{1/2}$$

where c_i and c_j are the concentrations and Z_i and Z_j the valencies of the cations and anions, respectively, N the Avogadro number, D the dielectric constant, k the Boltzmann constant, T the absolute temperature, and ε the electronic charge. Calculations of surface charge density from electrophoretic mobility measurements may result in an underestimate of the charge density because of problems of surface conductivity (*37*). For instance, the electrokinetic charge of Na^+-montmorillonite calculated by Marshall (*33*) from the electrophoretic mobility was 5.1×10^3 esu/cm^2. When calculated from published values (*38*) for a cation-exchange capacity of 100 me/100 g and the specific surface area of 750 m^2/g, the surface charge density is 3.8×10^4 esu/cm^2.

D. Particle Size Analysis

To overcome some of the difficulties associated with direct microscopic observation and with plate counting techniques, Santoro and Stotzky (*39,40*) have investigated the use of an electrical sensing zone particle analyzer (Coulter counter) to determine changes in particle size distributions resulting from sorptive interactions between microorganisms and clay minerals. A comparison was made of particle size distributions of individual component populations and those of mixed populations. When interactions between clays and microorganisms occurred, the particle size distribution of the mixture differed from the theoretical sum of the component populations.

14-3. OBSERVATIONS ON SORPTION OF MICROOGANISMS BY SOILS

The ability of different soils to sorb bacteria varies according to the mechanical composition of the soils, the species and growth conditions of the microorganisms, and the medium used for studying the sorption process. Minenkow (*41*) reported that for a wide range of soil types sorption of

Bacillus cereus var. *mycoides* and *Serratia marcescens* increased with increasing clay content (Fig. 14-1), and to some extent with increasing medium and fine silt. *Bacillus cereus* var. *mycoides* was more strongly sorbed than *S. marcescens* by most soils. It is obvious that relatively low amounts of clay resulted in a high percent sorption of the bacteria. However, Singh-Verma (*42*) failed to observe sorption of bacteria following the addition of 2% sterile loess soil to bacterial suspensions. Few bacteria are sorbed on normal sand

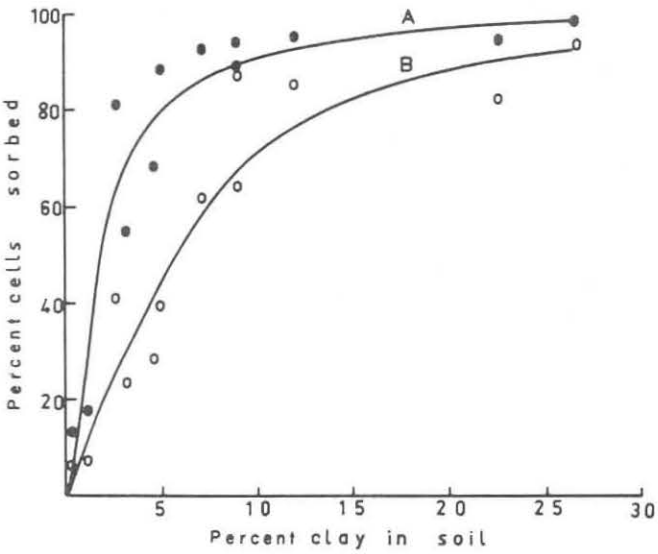

Fig. 14-1. Increase in the percent sorption of bacteria with increasing clay content of a range of soil types: (A) *B. cereus* var. *mycoides*; (B) *S. marcescens*. From Minenkow (*41*).

particles (*6*,*43*), although Karpinskaya (*7*) used finely ground quartz fractions to demonstrate that the percent of *S. marcescens* cells sorbed increased as the particle size decreased and as the quantity of each fraction added to a bacterial suspension increased (Table 14-1). Karpinskaya (*7*) observed the sorption of finely dispersed quartz particles on cells of *B. cereus* var. *mycoides* but not on other bacteria examined. The effects of cation saturation of the soil were considered by Peele (*8*). Sorption of *Azotobacter chroococcum* was negligible in Volusia silt loam saturated with monovalent cations and was highest in soil saturated with trivalent cations (Table 14-2).

The absolute number of cells sorbed by soil increases as the number of bacteria introduced increases, but the relative sorption capacity of the soil decreases (*6*). However, Rubentschik et al. (*44*) reported a zone of maximum sorption of *S. marcescens* by liman mud within the limits of $11–17 \times 10^6$

TABLE 14-1

Sorption of *S. marcescens* on Quartz Particles of Increasing Diameter Expressed as the Percentage of the Numbers of Cells Introduced (*7*)

Particle diameter of quartz, μ	Quantity of quartz fraction added to a bacterial suspension, g		
	0.02	0.25	1.00
	Percent sorbed		
<1.5	66	100	100
1.5–5.0	50	90	100
5.0–10	23	81	99
10–50	9	28	67
50–250	10	0	7
250–1000	0	0	0

cells per millilter of suspension, the percent sorption decreasing with higher or lower numbers of cells. With a constant number of bacteria, sorption increases with increasing soil levels until a maximum characteristic of the soil or the organism is attained (*6,7,44*).

An important factor influencing microbial ecology in soils must be the

TABLE 14-2

Effect of Cations Saturating the Exchange Complex of Volusia Silt Loam on the Sorption of *A. chroococcum* (*8*)

Saturating cation	Sorption, %
Li^+	1
K^+	4
Na^+	0
NH_4^+	1
Mg^{2+}	9
Ba^{2+}	35
Ca^{2+}	22
Mn^{2+}	61
Fe^{3+}	94
Al^{3+}	92
Original Volusia silt loam	22

TABLE 14-3
Sorption of Different Bacteria by a Loamy Soil (*46*)

Species of bacteria	Sorption, %
Bacillus cereus var. *mycoides*	95.5
Bacillus Ellenbachensis	57.5
Bacillus mesentericus	40.5
Pseudomonas liquefaciens	79.5
Serratia marcescens	95.4
Chromobacterium violaceum	70.0
Escherichia coli var. *communis*	11.5

selective sorption of certain microorganisms on soil particles. Numerous authors (*6–8,41,45–49*) have noted differences in the degree of sorption of different bacteria by soils or colloidal materials (Table 14-3 and Figs. 14-1 and 14-2). Eisenberg (*45*) and Bogopolskii (quoted by Krasil'nikov in Ref. *49*) claimed that gram-positive bacteria were more readily sorbed than gram-negative species, but this claim was not substantiated by other investigators (*6,7,44,46,48*). Strains of *Escherichia coli* were poorly sorbed by different soils

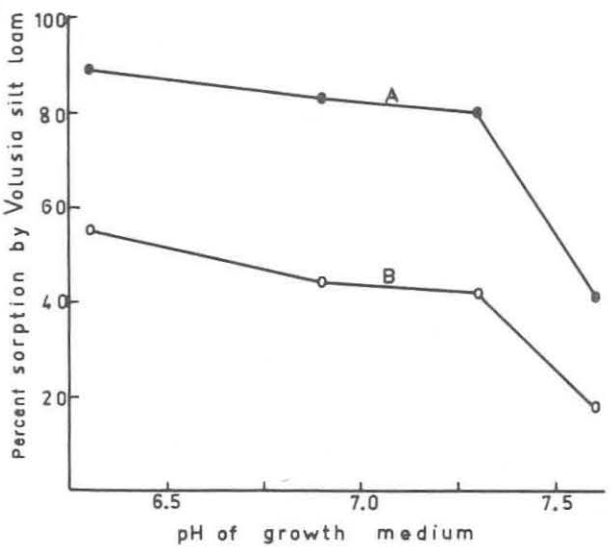

Fig. 14-2. Effect of pH of the growth medium on the sorption of bacteria on Volusia silt loam, indicating some alteration to the surface properties of the bacteria: (A) *A. chroococcum*; (B) *B. cereus* var. *mycoides*. From Peele (*8*).

(*6,7,49*) and by ion-exchange resins (*50*). Differences in the ability of various microorganisms to sorb onto glass surfaces have been reported (*11,51*). Peele (*8*) reported that the pH of the growth medium markedly influenced the sorption of cells of *A. chroococcum* and *B. cereus* var. *mycoides* by Volusia silt loam. As the pH of the growth medium increased, the percentage of cells sorbed by the soil decreased (Fig. 14-2). Eisenberg (*45*) had noted a similar effect of the pH of the growth medium on sorption of cells by soils. In a saline environment, Rubentschik et al. (*44*) and Waksman and Vartiovaara (*43*) found that muds contained bacteria whose degree of sorption is higher and desorption lower than that of bacteria isolated from water.

Tests reported by Chudiakow (*46*) indicate that when a soil appears saturated with a single organism (*S. marcescens*) sorption of a second organism (*B. cereus* var. *mycoides*) is prevented. Zvyagintsev (*25*), however, clearly demonstrated sorption of *B. cereus* var. *mycoides* cells on an anion-exchange resin previously saturated with cells of *S. marcescens*. He stresses the fact that the *S. marcescens* cells were not displaced by other cells in this test (i.e., sorption exchange did not occur). Rubentschik et al. (*44*) have presented evidence for sorption exchange when large numbers of *S. marcescens* were added to liman mud from a salt lake (Table 14-4). These figures represent approximately 1 additional cell desorbed for every 10 cells of *S. marcescens* sorbed.

TABLE 14-4

Exchange Sorption Following Addition of *S. marcescens* to 2.0 g of Liman Mud (*44*)

Treatment	No. *S. marcescens* cells sorbed	No. normal soil bacteria desorbed
Sterile water (10 ml)		3.00×10^5
Serratia marcescens (10 ml containing 4.8×10^6 cells)	4.175×10^6	7.25×10^5
Difference attributed to exchange sorption		4.25×10^5

This sorption of cells by soil particles is of great practical significance in attempts to count soil microorganisms. Rubentschik et al. (*44*) reported that desorption of *S. marcescens* from muds increased with time of shaking but the maximum number of cells desorbed was only about 6% of the total. The effectiveness of desorption and dispersion of microorganisms in soils varies tremendously with the soil type and the method used to desorb the organisms (*9,10,52,53*).

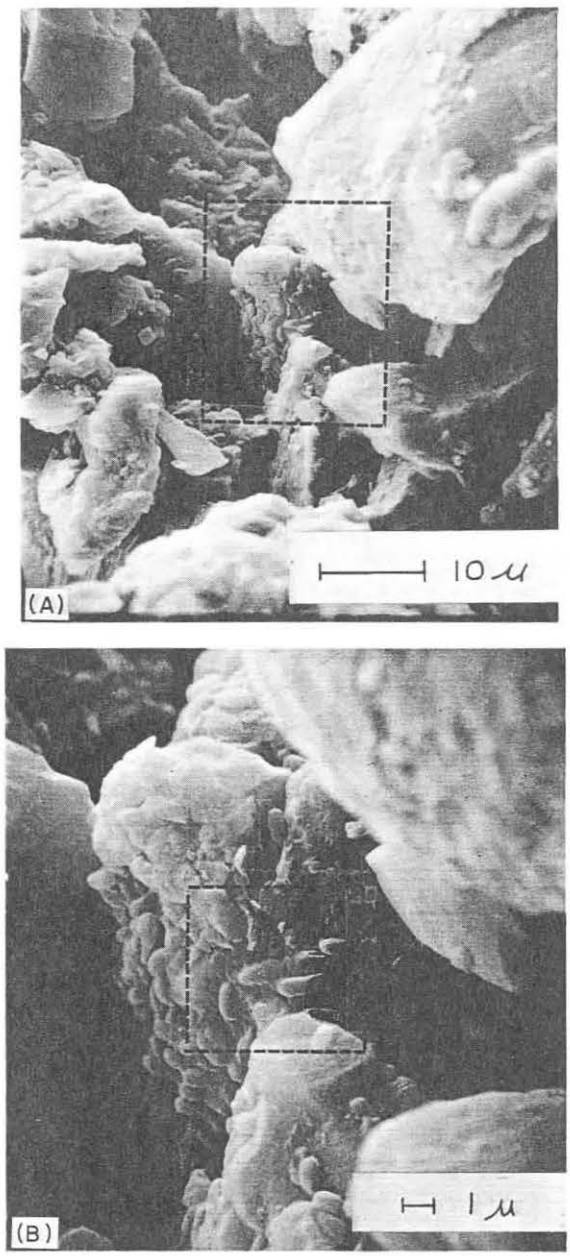

Fig. 14-3. Scanning electron micrographs of *B. cereus* cells on the surface of large soil particles. Area enclosed by dotted lines is the field of view at next higher magnification; (A) × 1550; (B) ×4700; (C) ×15,500. From Hagen et al. (*28a*), by courtesy of the American Society for Microbiology.

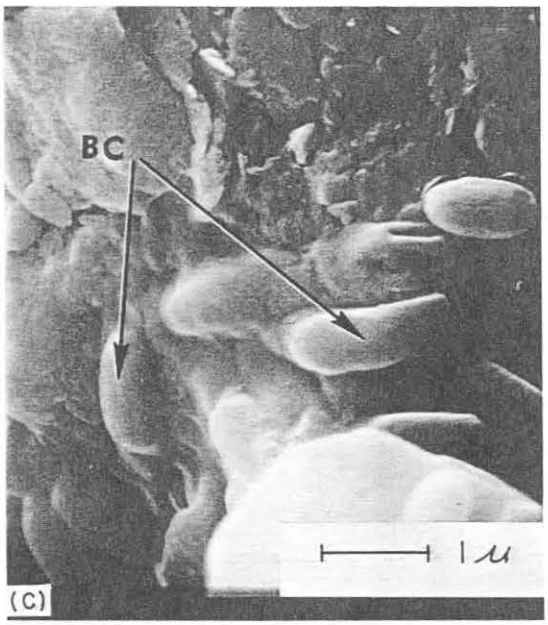

Fig. 14–3. (cont.)

14-4. TYPES OF INTERACTIONS BETWEEN SOIL PARTICLES AND MICROORGANISMS

Interactions between microorganisms and soil particles may be considered as three distinct processes (*17*). These are (1) the sorption of microorganisms onto the surfaces of large soil particles; (2) sorptive interactions between cells and soil particles of about bacterial size resulting in the formation of aggregates; and (3) the sorption of very small particles onto the surfaces of microorganisms (Figs. 14-3, 14-4, and 14-5).

A. Sorption of Cells on Large Soil Particles

Several recently developed techniques have provided some information on the distribution and orientation of microbial cells on the surfaces of large soil particles. In particular, the scanning electron microscope (*28,28a*) has provided a means of examining the surface of soil particles at very high levels of magnification (Fig. 14-3). The method is limited by the large numbers of specific organisms required for effective observation (*28a*), by dehydration and distortion of cells (*28*), and by the fact that the nature and degree of organization of the soil particles observed cannot be determined.

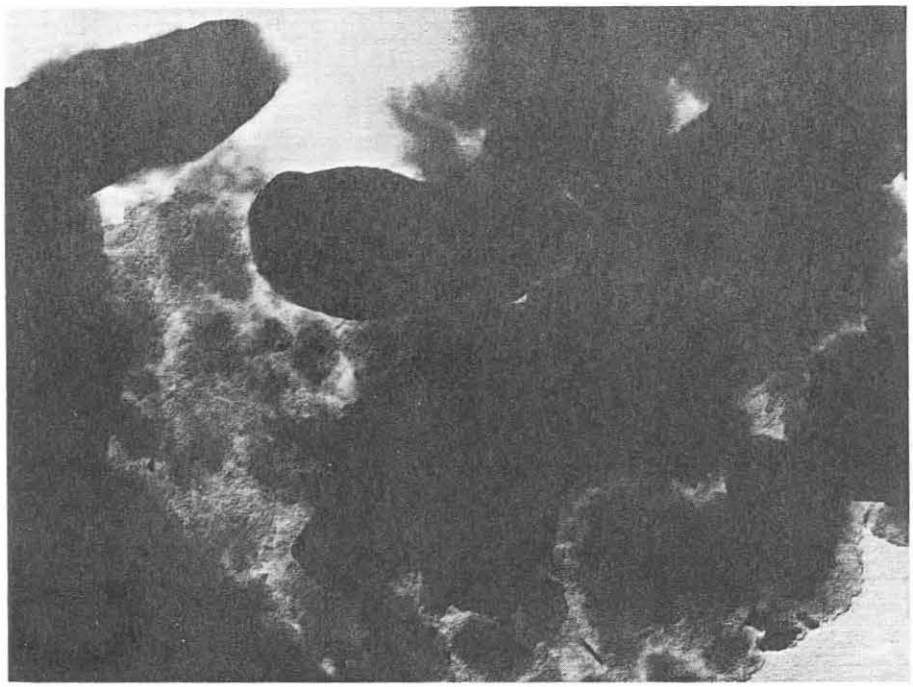

Fig. 14-4. Mutual sorption of bacterial cells and soil particles of a comparable size. Electron micrograph of freeze-dried suspension from a black earth soil. Gold-palladium shadowed (×27,000).

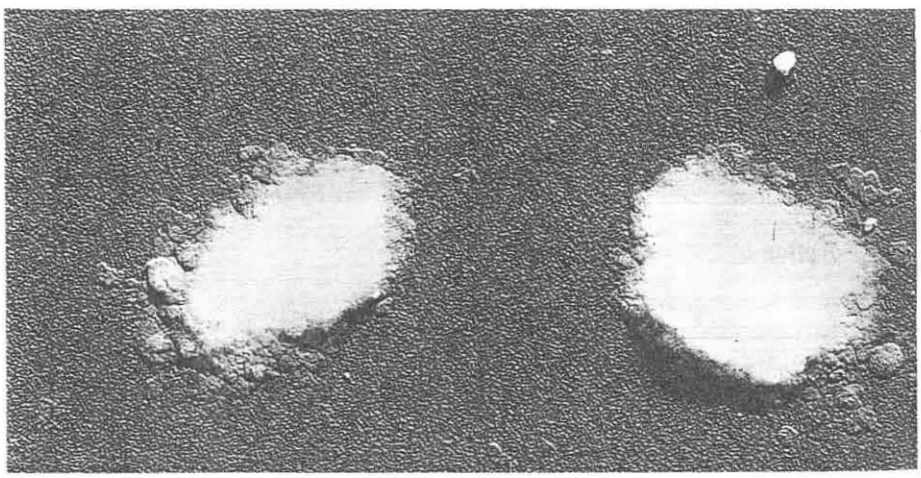

Fig. 14-5. Sorption of small clay platelets on bacterial cells. Electron micrograph of freeze-dried suspension from a black earth soil. Gold-palladium shadowed (×27,000).

Using normal transmitted UV light for studies on flurochrome-stained cells in soils, Burrichter (*54*) reported few cells on large soil particles and a maximum number sorbed on particles of 20- to 25-μ diameter. Zvyagintsev (*17*) used reflected illumination to show that the number of cells sorbed is directly proportional to the size of the particles in a humic-gley soil (Table 14-5), and attributed Burrichter's results to an inability to observe fluorochrome-stained cells on large particles using transmitted illumination. It is

TABLE 14-5

Distribution of Microorganisms on Particles of Humic-Gley Soil as Determined by Reflectance Fluorescence Microscopy (*17*)

Particle diameter, μ	No. cells sorbed per particle
1–2	1
4–5	4
10–12	8
20–25	29
40–50	110
100–110	300
400–500	10,100

possible, however, that the large particles examined by Zvyagintsev in the humic-gley soil may have been organic materials with surface charge characteristics different from those of the fine particles. Reflected illumination has been used by Gray et al. (*55*) to demonstrate that about 60% of bacteria in a sand dune soil are located on organic particles, which represent only 15% of the available, colonizable surface of the soil. They calculated that only 0.02% of the available surface area of the sand grains was colonized by bacteria. Uneven distribution of microorganisms on soil particles also has been observed by Zvyagintsev (*17*) and Krasil'nikov (*49*). Even on a single particle, the distribution of cells is far from homogeneous (*17,28,28a*). Although the degree of sorption of cells on large, relatively inert particles such as quartz may be low, the presence of coatings of clay (argillans) or organic matter (organans) on some large particles (*4*) may explain the uneven distribution of cells on the particles.

In view of the general lack of uniformity of soil particles, future studies of the sorption of microorganisms on large soil particles should include detailed information on the organization of the solid constituents of the soils being examined. Much of this information can be obtained by examination of thin sections of soils using a polarizing microscope. Modern developments in soil fabric analysis have been reviewed by Brewer (*4*). An example is given below

of some preliminary studies on a red earth soil (*56*) using this approach (Marshall, unpublished results). Comparisons of fields under normal transmitted illumination (Fig. 14-6A) and under crossed nicols (Fig. 14-6B),

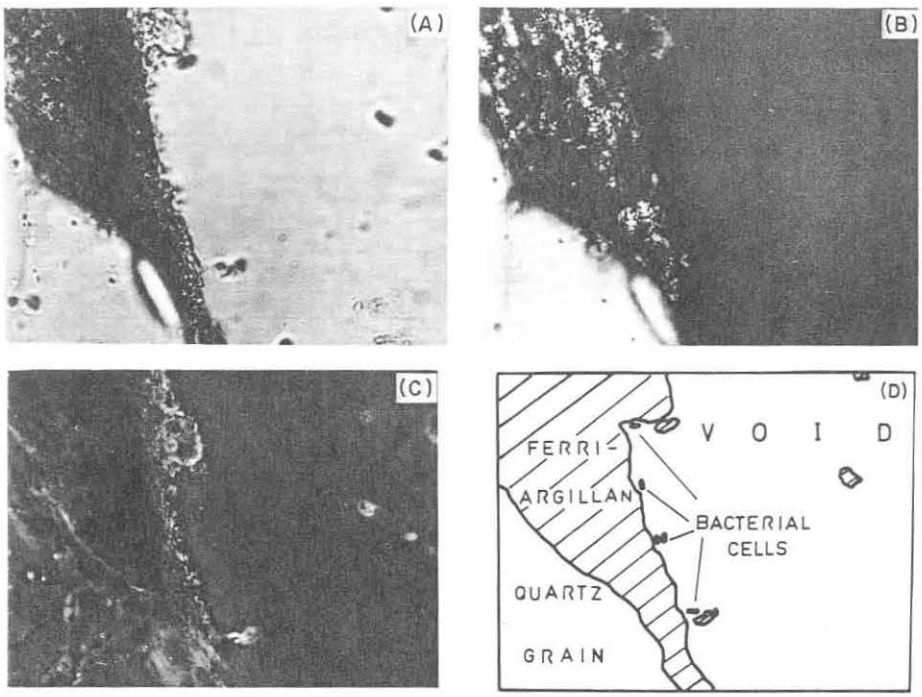

Fig. 14-6. Thin section of red earth soil. Oil immersion (×1,500): (A) bright field illumination; (B) crossed nicols; note the darkened void, brighter quartz grain and birefringent, oriented cutan of clay and iron oxide (ferriargillan); (C) phase contrast; note bacterial cells sorbed on the outer surface of the argillan; (D) diagrammatic interpretation of features observed in (A), (B), and (C).

permit the identification of various soil constituents and provides information on their general organization. Quartz grains, plant debris, mineral accumulations, voids (pores), and many other micromorphological features are readily identified. Most quartz gains in this soil are coated with a birefringent material identified as cutans of oriented clay and iron oxide (=ferriargillan). Once the organization of the solid constituents of the soil has been assessed, information on sorption of microorganisms may be obtained by examination of the same field by means of phase-contrast microscopy (Fig. 14-6C). Alternatively, samples of the same soil may then be examined in a more logical manner by some of the other techniques mentioned earlier.

This approach should provide a greater understanding of the distribution

of microorganisms in soils in relation to the arrangement of the solid phase of the soil. In particular, the distribution of organisms within soil peds may be examined in relation to the availability of organic materials in minute pores (*10,57*).

B. Aggregate Formation by Microorganisms and Soil Particles of Bacterial Size

Here we are concerned with aggregates resulting from sorptive interactions between microbial cells and particles of a comparable size (Fig. 14-4). Which of the two phases constitutes the adsorbent or the adsorbate probably varies with the size and number of the individual cells or particles. For such a situation, Krishnamurti and Soman (*30*) have suggested that the terms adsorbent and adsorbate are relative and have referred to this phenomenon as the *mutual sorption of colloidal particles*. Such mutual sorption has been observed microscopically (*7,8,58*). Electron micrographs of simple aggregates of cells and soil particles have been published (*27,59*).

The effects of different cations on aggregate formation by bacteria and soil particles have been stressed by Peele (*8*). Using *Azotobacter*, he has presented microscopic evidence for the lack of aggregate formation in a soil experimentally saturated with Na^+ ions, compared with obvious aggregate formation in the normal soil and in Al^{3+}-saturated soil. Using counting procedures, Peele demonstrated that sorption of bacteria by soil was either negligible, moderate, or strong depending on whether the soil was saturated with mono-, di-, or trivalent cations, respectively (see Table 14-2). Santoro and Stotzky (*39,40*) obtained similar results with pure clay preparations using the Coulter counter to determine aggregate formation. They showed that mutual sorption increased as the valency of the cations on the clays increased. When montmorillonite homoionic to monovalent cations was used, aggregate formation with bacterial cells occurred only at low pH levels. Sorption of bacteria was not influenced by the size, the motility, nor the Gram reaction of the bacteria nor by the size of the clay particles. Using a similar technique, Hattori (*60*) found no evidence for mutual sorption between Na^+-kaolinite and *E. coli* but has described a one cell–one particle complex between bacteria and Na^+-pyrophyllite. When pyrophyllite homoionic to di- and trivalent cations was used, the number of cells sorbed per clay particle increased with increasing cell concentration (*61*). Aggregate formation with yeast cells was found only in the presence of trivalent cations (*39*). Santoro and Stotkzy (*62*) have also used the Coulter counter to study the flocculation of clay minerals by various microbial metabolites.

An electrophoresis cell has been employed by Peele (*8*) to separate Volusia silt loam into positively and negatively charged fractions. Sorption of 90% was observed when *A. chroococcum* cells were added to the positively charged

fraction, but no sorption was observed for the negatively charged fraction. Oksentian (*29*) reported that values for the electrokinetic potentials were talc > kaolin > animal charcoal, yet their relative ability to sorb yeasts and lactic acid bacteria was in the reverse order. Thus the degree of sorption increased with decreasing negative charge on the particles. The electrophoretic mobilities of bacterial cells and kaolin, both separately and in mixtures, have been determined by Krishnamurti and Soman (*30*). The results (Table 14-6) suggest that cells are sorbed onto the surface of kaolin particles

TABLE 14-6

Electrophoretic Mobilities of Bacteria and Kaolin, Both Separately and in Mixture[a]

Treatment	Electrophoretic mobility, μ/sec/V/cm
Kaolin	−4.58
Bacillus subtilis	−0.96
Mixture of kaolin and *B. subtilis*	−0.57

[a] Recalculated from the data of Krishnamurti and Soman (*30*).

and completely cover the surface, although it is difficult to explain why the mobility of the aggregates was lower than that of the cells.

According to Krishnamurti and Soman (*30*), the curves obtained by plotting numbers of cells sorbed per gram of adsorbent against increasing initial numbers of bacteria are distinctly S-shaped. They have presented an adsorption equation which provides a more reliable fit to their data than the classic equations of Freundlich or Langmuir. They also reported that the degree of sorption of bacteria by charcoal increases as the temperature decreases (Fig. 14-7), an observation that is consistent with an adsorption process.

C. Sorption of Small Particles by Bacterial Cells

Karpinskaya (*7*) reported the sorption of very fine quartz particles by cells of *B. cereus* var. *mycoides* but not by cells of *S. marcescens*. This phenomenon cannot be observed by normal light microscopy when very fine colloidal particles (<0.1-μ diameter) are used. Indirect evidence for such sorption based on the interference by colloidal montomorillonite with the fluorescent image of fluorochrome-stained cells has been presented (*23*). Jackson et al. (*27*) and Marshall (*23*) have published electron micrographs that show the sorption of colloidal clay particles to the surface of bacterial cells.

The first detailed study of this phenomenon was that by Lahav (*31*), who

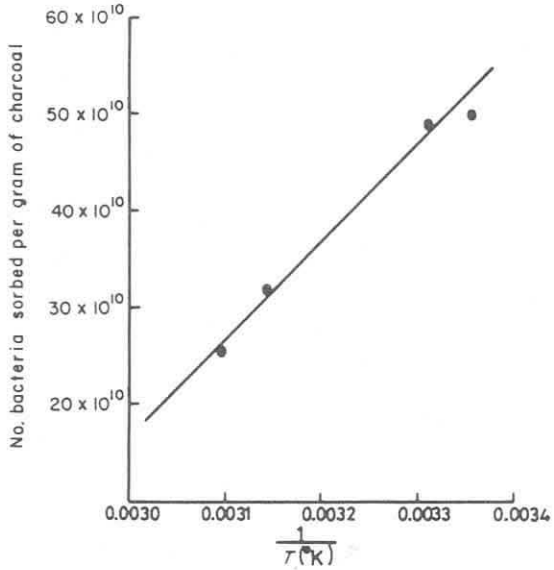

Fig. 14-7. Relationship between the degree of sorption of bacteria by charcoal and the reciprocal of the absolute temperature. From Krishnamurti and Soman (*30*), by courtesy of the Indian Academy of Science.

used a microelectrophoretic technique. He demonstrated that sorption of Na^+-montmorillonite by cells of *B. subtilis* was a reversible process. The electrophoretic mobility of cells was shown to increase with increasing montmorillonite concentration, the increase being greater at pH 3.8 than at higher pH values. At pH 8.0 no increase in cell mobility was observed on addition of clay. Lahav attributed this pH effect to a higher positive charge density on the edge of the clay particles at lower pH values. The mobility of cells in montmorillonite suspensions also depended on the ionic strength and nature of the anion of the electrolyte employed. Lahav found two populations of *B. subtilis* in his experiments. The mobility of one group increased with increasing clay concentration, while the mobility of the second group remained unchanged. The latter group apparently did not sorb clay particles. Contrary to this result, Marshall (*23*,*33*) found that all cells of various species of *Rhizobium* and of a different strain of *B. subtilis* gave a uniform response to the addition of Na^+-montmorillonite or Na^+-illite.

The increase in mobility of cells with increasing clay concentration indicates a progressive envelopment of the cell surface by the smaller clay particles. This is well illustrated by a comparison of the effects of montmorillonite and illite, both made homoionic to sodium, on cell mobility (Fig. 14-8). In the presence of montmorillonite, the mobility of all-strains of *Rhizobium* increased

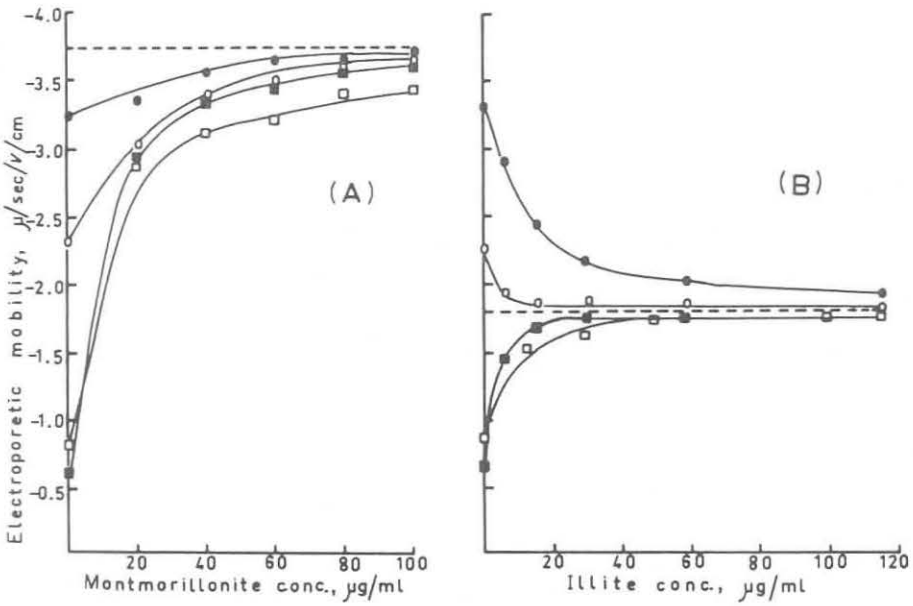

Fig. 14-8. Changes in the observed electrophoretic mobility of bacteria with increasing clay concentration: (A) Na^+-montmorillonite; (B) Na^+-illite. ●——●, *Rhizobium trifolii* SU298D; ○——○, *R. trifolii* TA1; ■——■, *R. lupini* UT12; □——□, *R. trifolii* SU297B; - - - - -, mobility of montmorillonite (A) or illite (B) particles. From Marshall (*23*), by courtesy of Cambridge University Press.

to a level approximating that of the clay itself, regardless of the initial mobilities of the different cultures (*33*). Similar results were obtained in the presence of illite with cultures of characteristically low mobility. With cultures exhibiting higher electrophoretic mobilities than that of the illite, however, their mobilities decreased with increasing clay concentration until they approximated that of the illite (*23*). Although bacterial cells are being observed under the microscope, it appears that the measured electrophoretic mobility is actually a characteristic of clay particles enveloping the surface of the cells.

Using the limiting mobility method of Nevo et al. (*63*), Lahav (*31*) calculated that the amount of montmorillonite sorbed by *B. subtilis* in a sodium phosphate solution ($I = 0.05$) was approximately 2×10^{-6} μg per cell, while in a sodium chloride solution of the same ionic strength the amount sorbed exceeded 10×10^{-6} μg per cell. Somewhat similar levels in sodium chloride ($I = 0.05$) have been obtained by Marshall (*23,33*) for the sorption of montmorillonite and illite on various species of *Rhizobium* (Table 14-7). There

TABLE 14-7

Saturation Levels of Sorbed Na^+-Montmorillonite and Na^+-Illite per Cell and per Unit of Cell Surface in Relation to Some Properties of Isolates of *Rhizobium* (*23,32,33*)

Organism	Relative growth rate	Surface ionogenic groups	Electro-kinetic charge esu/cm²	Surface area of cells, μ^2	Montmorillonite sorbed		Illite sorbed	
					μg/cell ($\times 10^{-6}$)	$\mu g/\mu^2$ ($\times 10^{-6}$)	μg/cell ($\times 10^{-6}$)	$\mu g/\mu^2$ ($\times 10^{-6}$)
Rhizobium lupini UT2	Slow	Carboxyl	−3647	4.58	2.29	0.50	—	—
Rhizobium lupini UT12	Slow	Carboxyl	−3260	9.24	4.70	0.51	4.90	0.53
Rhizobium japonicum QA372	Slow	Carboxyl	−3868	5.08	2.90	0.57	—	—
Rhizobium trifolii TA1	Fast	Carboxyl	−4374	6.82	4.00	0.59	4.10	0.60
Rhizobium trifolii SU297A	Fast	Carboxyl-amino	−3062	7.91	2.50	0.32	—	—
Rhizobium trifolii SU297B	Fast	Carboxyl-amino	−2751	5.58	1.60	0.29	1.45	0.26
Rhizobium trifolii SU298D	Fast	Carboxyl-amino	−5020	8.17	2.03	0.25	2.30	0.28
Rhizobium leguminosarum TA101	Fast	Carboxyl-amino	−3462	11.25	2.95	0.26	—	—

appears to be no obvious relationship between the surface charge density of the cells and the amount of clay sorbed by the various bacteria. A distinct relationship is apparent between the amount of clay (either illite or montmorillonite) sorbed per unit area of cell surface and the nature of the surface ionogenic groups of the bacteria. Marshall (*32*) observed that some rhizobia possessed characteristic carboxyl-type surface ionogenic groups, exhibiting zero electrophoretic mobility at pH 2.0 and a constant negative mobility between pH 4.0 and 10.7. Other types showed slight positive mobility at pH 2.0, a constant negative mobility between pH 4.0 and 9.2, and a marked increase in negative mobility between pH 9.2 and 10.7. It was suggested that the surfaces of these bacteria contain a predominance of acidic (carboxyl) groups along with some basic (amino) groups. Cells with an exclusively negatively charged surface (carboxyl type) sorb approximately twice as much clay as those with a complex carboxyl-amino surface (Table 14-7). Sorption of the clays appears to be independent of the surface charge properties of the clays since virtually identical amounts of both illite and montmorillonite are sorbed per cell.

It is unlikely that any one of these three forms of association between microorganisms and soil particles occurs exclusively in many natural soils. A wide range of particle sizes exists in normal soils and, consequently, a variety of sorptive interactions should occur. Electron microscope studies of suspensions of a soil described as a black earth by Loveday (*64*) have revealed both sorption of fine particles on cells and aggregate formation between cells and particles of comparable size (Figs. 14-4 and 14-5). However, if much of the colloidal materials of a particular soil occur as cutans, then it might be expected that most cells will be sorbed to large, colloid-coated particles. Examination by electron microscopy of a suspension of a red earth soil, in which clays exist as ferriargillans (see Fig. 14-6), showed few cells with clay sorbed to their surfaces and few aggregates of cells and soil particles.

Hattori (*61*) has attempted to describe some of the interactions between cells and soil particles in the following equations:

$$\text{Type 1:} \qquad m\text{B} + n\text{C} \rightleftharpoons \text{B}m\text{C}n$$

$$\text{Type 2:} \qquad m\text{B} + n(\text{CM}) \rightleftharpoons \text{B}m(\text{CM})n$$

where B is a bacterial cell, C a clay particle, CM a clay particle saturated with di- or trivalent cations, m the number of bacterial cells and n the number of clay particles. Examples of type 1 are interactions in which many small particles are sorbed on a bacterial cell ($m = 1$, $n > 1$), and in which one cell is sorbed by one Na^+-pyrophyllite particle ($m = 1, n = 1$). A type-2 interaction occurs when aggregates are formed between cells and clays saturated with di- or trivalent cations ($m > 1$, $n > 1$). Hattori has proposed that when

the value of m or n is 1, the association should be termed a bacterium–clay complex. When the values of both m and n exceed 1, the association should be termed a bacterium–clay aggregate.

14-5. MECHANISM OF SORPTIVE INTERACTIONS BETWEEN SOIL PARTICLES AND MICROORGANISMS

It seems likely that the mechanisms of sorption of microorganisms by large particles or by particles of bacterial size are similar. Consequently, these two types of interactions are discussed together.

A. Sorption of Microorganisms by Particles of Bacterial Size or Larger

In reviewing studies on sorption of bacterial cells by soils, Dikusar (*65*) concluded that electrostatic attraction played little part in the sorption process since both the particles and the bacteria exhibit a net negative charge at normal soil pH levels. Similar views have been expressed by other workers (*30,46*). Bacterium–soil particle interactions are obviously very complex and several different sorptive mechanisms are likely. Both microorganisms and soil particles, such as clays and organic materials, can possess positively charged sites on an otherwise predominantly negatively charged surface. Peele (*8*) separated a positively charged fraction from a soil and found that it sorbed bacterial cells very strongly. The fact that different microorganisms vary in their ability to sorb onto soils (6–8,*41,45,46*), clays (*39,47,48*), ion-exchange resins (*25,50*), and glass (*11,51*) emphasizes the complexity of the problem. Zvyagintsev (*17*) has noted that cells of *S. marcescens* attach by their sides to most soil particles. On some particles, however, the cells are attached by their pole. This suggests an uneven distribution of positive and negative charges on the surface of the bacterial cells. The ability of a microorganism to be sorbed varies with the conditions under which the organism is cultured (*8,11*). Dead bacterial cells appear to behave in a fashion similar to living cells (*7,39*).

Oksentian's (*29*) finding that the degree of sorption increased as the net negative charge on the adsorbent decreased suggests that coulombic forces are important in these sorption processes. Surfaces with a lower net negative charge may have some positively charged sites at which sorption of negatively charged cells may occur. On the basis of electrophoretic measurements, Santoro and Stotzky (*34,35*) have suggested that the isoelectric point of bacterial cells in soils may be shifted to higher pH values than found *in vitro*. They consider that this might be important in sorptive interactions between cells and negatively charged clay particles. More evidence for the role of electrostatic attraction is provided by investigations on the effects of cation saturation of soil particles on sorption of bacteria (*8,39,60,61*). According to

Santoro and Stotzky (*39*), sorption between microbial cells and clay minerals, in accordance with the Schultz–Hardy rule, increased when the electrokinetic potential of the particles was decreased by polyvalent cations. They suggest that under such conditions hydrogen bonding and London–van der Waal's forces may be involved in the sorptive interactions. A greater degree of sorption at lower pH values was attributed by Santoro and Stotzky (*39*) to a net positive charge on the bacterial cells, but this explanation does not apply to cells with only carboxyl-type surface ionogenic groups (*32,66,67*). The positive charge density on the edge of clay particles also increases at lower pH values (*38*) and this may be of significance in such sorptive processes. Hattori (*61*) has suggested that the higher degree of sorption by clays saturated with polyvalent cations may result from a "cationic bridge" formed between the clay and bacterial surfaces.

This effect of polyvalent cations cannot account for the sorptive interactions observed between microorganisms and materials with a negligible cation-exchange capacity, such as glass (*51,68,69*) or finely ground quartz (*7*, see Table 14-1). Various workers investigated the phenomenon of inorganic and organic nutrient accumulation at the surface of chemically clean glass (*51, 68–70*) and considered that this accumulation of nutrients was independent of and precedes bacterial development on the surfaces. As pointed out by Tyler and Marshall (*71*), positively charged molecules could be attracted to negatively charged surfaces, such as glass or sand grains, resulting in the formation of a diffuse double layer. This layer might provide electrostatic attraction for negatively charged bacteria and at the same time a favorable concentration of nutrient materials. Once attracted to the surface, the cells may produce some holdfast material to give a more permanent binding to the surface (*51*). In fact, ZoBell (*51*) has observed that most sessile, strongly sorbing bacteria in aquatic environments appear to secrete mucilaginous holdfast material. Attachment of some organisms such as *E. coli*, is superficial (*69*), since they are readily detached by shaking. This probably indicates that such cells lack the ability to produce a suitable mucilaginous material. Beutelspacher (*59*) has suggested, on the basis of an electron microscope examination of soils, that bacterial flagella may provide some means of binding cells to soil particles. Pili (or fimbrae) on bacterial surfaces are more likely to be important in sorption processes since they are very numerous when present and they substantially alter the net surface charge density of cells (*72*). Nikitin (*26*) has observed pili on numerous soil microorganisms, particularly those from enrichments in nutrient-deficient media.

B. Sorption of Small Particles by Microorganisms

Lahav (*31*) has considered the question of the orientation of clay particles sorbed by *B. subtilis* cells. Because of their platelike structure the particles

may be oriented in three different ways: (1) *face-to-face sorption* with the broad particle (platelet) surface facing the bacterial surface; (2) *edge-to-face sorption* with the edge of the platelets facing the bacterial surface; (3) *mixed sorption* with platelets sorbed in both ways.

The net charge on these platelets is negative, but at pH values below 7 the edges of the platelets are positively charged (*38*). Lahav suggests that all three types of orientation could occur, depending on the experimental conditions. He suggested that edge-to-face sorption probably occurs in a sodium chloride solution, resulting in greater amounts of clay sorbed than in sodium phosphate or hexametaphosphate solutions in which most of the edge positive charges are neutralized and any sorption is probably face-to-face. The reason for Lahav's claim that mixed sorption occurs in solutions of low pH or low ionic strength is not clear in view of the lack of information on the surface charge properties of his strain of *B. subtilis* under such conditions. Likewise, the reason for the lack of sorption of montmorillonite observed at pH 8.0, at which the edge positive charge is suppressed, is not clear if face-to-face sorption can occur.

A comparison of the sorption of clays on species of *Rhizobium* with only acidic (carboxyl) surface groups with other species possessing a predominance of acidic and some basic (amino) groups (*32*) has been reported by Marshall (*23,33*). Cells with a simple carboxyl surface sorb approximately twice as much Na^+-montmorillonite or Na^+-illite as cells with a complex carboxyl-amino surface (Table 14-7). A predominantly edge-to-face association between the clay platelets and cells with a carboxyl surface was suggested (*23,33,73*), since the positively charged edges of the clay would be attracted to the negatively charged surfaces of the cells. This type of orientation would account for the very large amounts of clay sorbed per unit area of cell surface (*33*). Sorption of clays by cells of the carboxyl-amino type should be mainly by an edge-to-face orientation. The effect of the amino groups has been considered as follows (*33,73*): (1) A diffuse distribution of the positively charged amino groups resulting in a less efficient edge-to-face stacking of the platelets because of repulsion effects; and (2) A nondiffuse (localized) distribution of the amino groups resulting in some face-to-face sorption of the platelets.

Preliminary studies on the effect of treatment of Na^+-montmorillonite with sodium hexametaphosphate (*73a*) lend support to the suggestion that some face-to-face sorption occurs on the carboxyl-aminotype cells (Table 14-8). Cells with carboxyl-type surfaces apparently do not sorb hexametaphosphate-clay (HMP-clay) since no increase in the electrophoretic mobility of the cells was observed in the presence of HMP-clay. Edge-to-face orientation is prevented by neutralization of the positive charges at the edge of the platelets by HMP. However, the elctrophoretic mobilities of cells with carboxyl-amino

TABLE 14-8

Influence of Na^+-Montmorillonite (100 μg/ml), in the Presence and Absence of Hexametaphosphate (HMP), on the Electrophoretic Mobility of *Rhizobium* Isolates with Different Surface Ionogenic Properties[a]

Organism	Surface ionogenic groups	Without clay	Na^+-montmorillonite		Na^+-montmorillonite + HMP	
		$\bar{v}$	$\bar{v}$	$\Delta\bar{v}$	$\bar{v}$	$\Delta\bar{v}$
Rhizobium lupini UT2	Carboxyl	−1.52	−3.36	−1.84	−1.50	+0.02
Rhizobium japonicum QA372	Carboxyl	−1.52	−3.52	−2.00	−1.51	+0.01
Rhizobium trifolii TA1	Carboxyl	−2.21	−3.50	−1.29	−2.27	−0.06
Rhizobium trifolii SU297A	Carboxyl-amino	−1.01	−3.29	−2.28	−1.66	−0.65
Rhizobium trifolii SU297B	Carboxyl-amino	−0.80	−3.60	−2.80	−1.85	−1.05
Rhizobium leguminosarum TA101	Carboxyl-amino	−1.08	−3.25	−2.17	−1.85	−0.77
Rhizobium leguminosarum UT11	Carboxyl-amino	−2.53	−3.75	−1.22	−3.25	−0.72

[a] $\bar{v}$ = Electrophoretic mobility (μ/sec/V/cm), and $\Delta\bar{v}$ = (mobility in presence of clay) − (mobility in absence of clay).

surfaces do increase in the presence of HMP-clay, although not to the same extent as in the presence of normal Na^+-montmorillonite. This suggests a limited amount of face-to-face association between the negatively charged face of the clay platelets and the positively charged amino groups on the bacterial surface. These interactions are represented diagrammatically in Fig. 14-9. The mobility of HMP-clay alone was similar to that of untreated Na^+-montmorillonite.

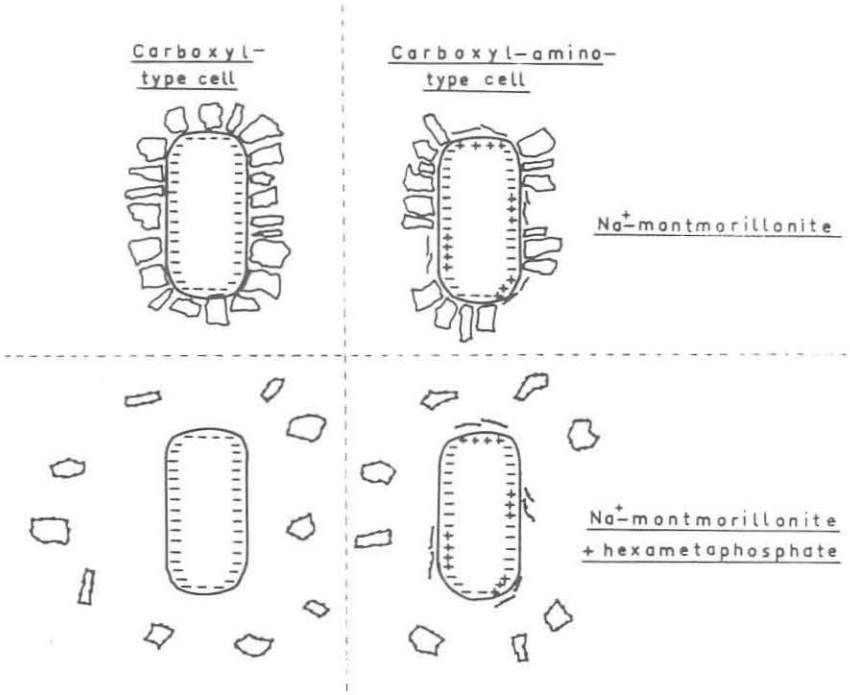

Fig. 14-9. Diagrammatic representation of the proposed edge-to-face association between Na^+-montmorillonite platelets and the negatively charged surface of a carboxyl-type cell and the lack of sorption of HMP-montmorillonite on this cell type. Both edge-to-face and face-to-face sorption of Na^+-montmorillonite are shown on the carboxyl-amino-type cell, but only face-to-face sorption where the positive charges at the edge of platelets are neutralized by HMP.

The work reported on the sorption of fine clay particles on bacterial cells concerns only clays homoionic to monovalent cations. Lahav (*31*) has briefly mentioned that montmorillonite homoionic to calcium reduces the electrophoretic mobility of bacterial cells.

14-6. IMPLICATIONS OF SORPTIVE INTERACTIONS ON THE BEHAVIOR OF MICROORGANISMS IN SOILS

A. Microbial Metabolism

An understanding of the effects of the solid phase of soils on the metabolic activity of microorganisms and on the catalytic activity of extracellular enzymes produced by these organisms is of fundamental importance to the science of soil biochemistry. McLaren (*74*) has stressed that an entirely new form of chemical kinetic equations is required to handle enzyme reactions in the sorbed state. The effects of sorption reactions on enzyme activity in soils has been thoroughly considered by Skujiņš in Volume 1 of this treatise (*75*) and reviewed by McLaren and Peterson (*150*).

It might appear at first glance that sorption of microorganisms by soil particles is of benefit to the organisms. The fact that particles can act as a surface for the concentration of nutrients from dilute solutions (*51,68–70,76*) could result in a stimulation of the activity of sorbed microorganisms. However, the literature is full of apparently conflicting reports of both stimulatory and inhibitory effects of solid particles on microbial activity (*6–8,10,29,44,46,65,77–98a*).

Many authors have noted a suppression of oxygen uptake or carbon dioxide evolution by microorganisms in the sorbed state with glucose or other carbon substrates (*6–8,29,46,81,92–96*). Dianowa and Woroschilowa (*6*) reported that the inhibition of carbon dioxide evolution was less with the weakly sorbed *E. coli* var. *communis* than with more strongly sorbed species. Karpinskaya (*7*) suggested that the observed suppression might result from the death of bacteria following sorption, but this point has not been verified by other investigators (*29,44,90*). A correlation between percent sorption and the degree of retardation of carbon dioxide evolution was reported by Peele (*8*). Soil saturated with trivalent cations showed strong sorption suppression of metabolic activity, while soil saturated with monovalent cations showed slight sorption and negligible inhibition of carbon dioxide evolution (Table 14-9). Lahav and Keynan (*84*) found that low concentrations of colloidal montmorillonite inhibited respiration of *B. subtilis* and resulted in aggregation of the bacterial cells. Respiration was stimulated at high montmorillonite concentrations and this was accompanied by a complete dispersion of the bacteria in suspension. Attapulgite did not influence respiration unless the suspension was shaken, when aggregation of cells was observed and the respiration rate was reduced. The respiriatory activity of bacteria sorbed on an anion-exchange resin (Dowex 1) was markedly reduced, and the pH optimum for sorbed cells was about 1 unit higher than that for free cells (*92–95*). Hattori and Hattori (*95*) have also reported differences in the respiratory

TABLE 14-9
Effect of Various Soil Treatments on Carbon Dioxide Evolution by *A. chroococcum* Using Glucose as Substrate[a]

Soil treatment	Sorption of bacteria, %	Inhibition of CO_2 evolution after 3 hours, %
Fe^{3+}-saturated Volusia silt loam	97	96
Al^{3+}-saturated Volusia silt loam	96	88
Ontario loam	90	78
Volusia silt loam	70	44
NH_4^+-saturated Volusia silt loam	5	0
Na^+-saturated Volusia silt loam	3	0

[a] Recalculated from the data of Peele (*8*).

response by free and sorbed cells over a range of temperatures. Respiration of fungi was not affected by montmorillonite or kaolinite at concentrations below 4% (*88*). Inhibition was observed at levels above 4% for montmorillonite and above 40% for kaolinite. The degree of inhibition was related to the viscosity of the suspensions, probably because of a reduction in the oxygen diffusion rate.

Stimulation of respiration by microorganisms utilizing carbon substrates in the presence of solid particles has been reported (*79,85–91*). Some clays, such as kaolinite, were found to have little effect on respiration of bacteria (*85–87*), while the 2 : 1-type clays, especially montmorillonite, very effectively stimulated respiration (Fig. 14-10). This differential effect resulted from the ability of the 2 : 1 clays to maintain the pH of the system at a level favorable for respiration. Using homoionic montmorillonites, Stotzky (*86*) found that the relationship between respiratory stimulation and saturating cation was Na > Ca > Mg > K > H. This order appears to reflect the relative basicity of the cations (except for potassium), rather than valence, ease of replacement from the exchange complex, or the ability to replace other cations from the complex. Stotzky (*87*) also reported that respiratory activity was related to the cation-exchange capacity and surface area, but not the particle size, of the clays.

Other reported effects of particulate materials on metabolic activities of microorganisms are stimulation of uric acid decomposition (*98*), suppression of adenine decomposition (*98*), stimulation (*77,80,99*) or suppression (*100*) of nonsymbiotic nitrogen fixation, a stimulation (*82,101,102*) or suppression (*44,82,100,103,104*) of ammonium oxidation, suppression (*83*) of nitrite oxidation, stimulation (*44,96*) of sulfate reduction, and no effect on thio-

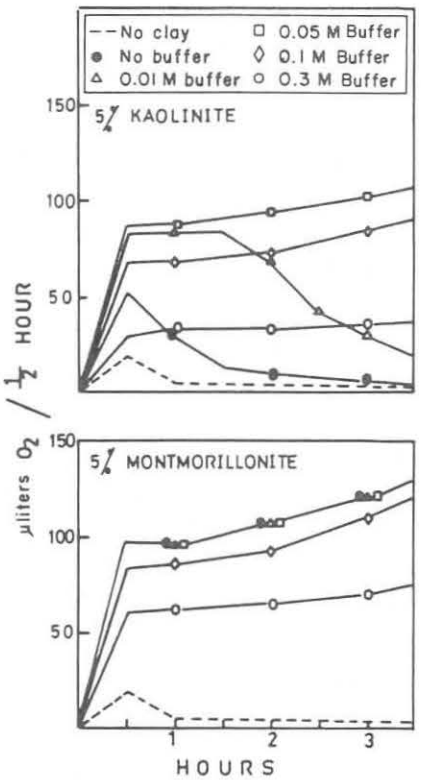

Fig. 14-10. Effect of buffer concentration on respiration of *Pseudomonas striata* in the presence and absence of kaolinite or montmorillonite. From Stotzky and Rem (*85*), by courtesy of the National Research Council of Canada.

sulfate oxidation (*44*). McLaren and Skujiņš (*83*) found that the pH for half-maximum nitrification rate is about 0.5 units higher in soil than in solution culture.

It is impossible to reconcile all of these results with any one mechanism that has been proposed. Stotzky (*86*) has established that buffering of the pH by 2 : 1-type clays results in a stimulation of bacterial respiration, but sorption by natural soil particles often results in an inhibition of respiration. Most of these studies have been carried out using glucose, a substrate poorly sorbed by clays (*105,106*). Peele's (*8*) results (see Table 14-9) suggest that in the soils he used sorbed cells are unable to metabolize glucose. One possible explanation is that substances inhibitory to bacteria are sorbed onto the same surfaces as the cells. The proposal by ZoBell (*51*) that small particles sorbed on the surface of cells may reduce bacterial activity might be relevant to this

problem. An envelope of clay around bacterial cells (*23*) may reduce or prevent the diffusion of nutrients to the cell surface. Even the movement of water would be restricted under these conditions, since it is so firmly bound (*107,108*) by the numerous clay platelets surrounding the cells. It is possible that in the soils studied by Peele and others that the cells are sorbed onto large particles and, in turn, small clay or organic colloidal particles sorb onto the surface of the cells. Yamagishi and Furusaka (*97*) have suggested that sorption of calcium phosphate gel on the surface of bacterial cells impairs the respiratory activity of the cells. Stimulatory effects of sorption interactions have been attributed to the concentration of essential nutrients at the solid–liquid interface (*51,68–70,76*).

The despression of nitrifying activity of cells sorbed on soils or cation-exchange resins has been explained by McLaren and Skujiņš (*83*) in terms of a repulsion of nitrite ions from the negatively charged surface of clays or resins to an extent sufficient to influence their availability to bacteria sorbed to the solid surfaces. According to McLaren and Skujiņš, the higher pH optima observed in soils and on cation-exchange resins result from a concentration of hydrogen ions at the solid–liquid interface. Consequently, a higher pH is required in the bulk solution of a solid–liquid system to achieve a hydrogen ion concentration at the interface equal to that found in a purely liquid medium (*109*). The opposite effect has been observed, however, by Hattori and Hattori (*95*) with cells sorbed on to a cation-exchange resin (Dowex 50). The pH optimum for glucose oxidation for sorbed cells was about 1 unit lower than for free cells. However, Hattori and Furusaka (*92–94*) have observed pH optima for the oxidation of a range of carbon substrates of about 1 unit higher for cells sorbed on an anion-exchange resin (Dowex 1) than for free cells.

It is obvious that more intensive studies on these aspects of metabolism of microorganisms in the sorbed state in soils or in model systems are required before satisfactory information can be obtained to explain the various inhibitory and stimulatory phenomena that occur in the complex environment of natural soils.

B. Microbial Growth

Although Karpinskaya (*7*) suggested that sorption of bacteria to soil particles may result in the death of appreciable numbers of cells, more recent work indicates that cells are able to multiply quite readily and that growth is often stimulated by the presence of solid particles (*29,43,51,68,69, 76,80,90,101,110–112*). The stimulatory effects of soil colloids on bacterial growth led to suggestions (*100,101,110*) that cultures be grown and maintained in synthetic soils or media containing colloidal clays. Conn and Conn (*110*) found that various clays stimulated bacterial growth and that samples of

montmorillonite made homoionic to various cations also stimulated growth. Least stimulation was found with NH_4^+- and Ca^{2+}-saturated montmorillonites, a result that does not agree with the stimulatory effects of similar clays reported by McCalla (*113*).

Stimulatory effects generally have been attributed to the accumulation of inorganic or organic nutrients at the solid–liquid interface (*51,68–70,76*). Heukelekian and Heller (*69*) clearly showed that the increased surface area provided by fine glass beads provided a site for the concentration of nutrients from a dilute glucose plus peptone medium. In the presence of beads, *E. coli* was able to grow in dilute media that did not support growth in the absence of beads (Fig. 14-11). At higher nutrient concentrations, the organism was less dependent on the beads as a concentrating surface. This phenomenon of nutrient concentration as well as that of selective sorption (*11*) of microorganisms at glass surfaces suggests a need for caution in the application of glass microbead model systems (*114–116*) to studies of the behavior of soil microorganisms.

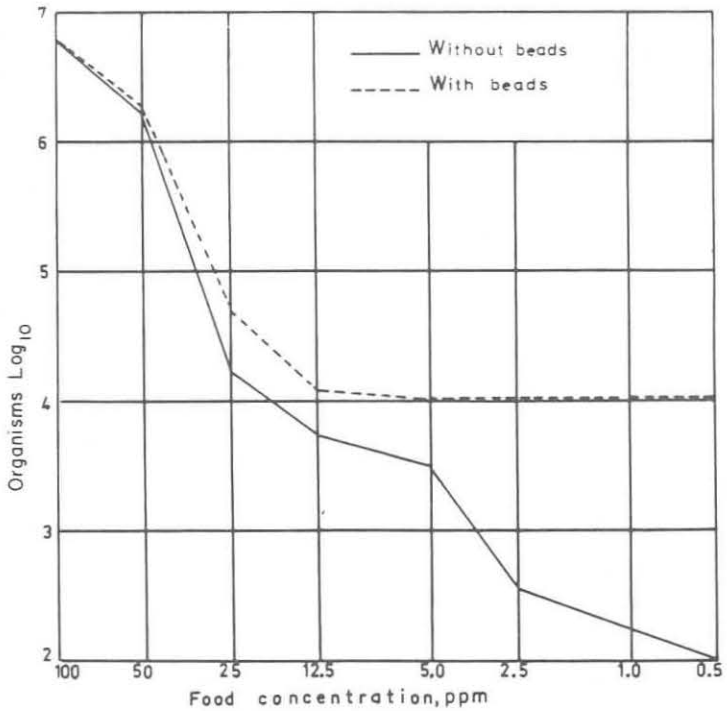

Fig. 14-11. Final numbers of *E. coli* found after 72 hours incubation in a range of concentrations of a glucose plus peptone medium in the presence or absence of glass beads. From Heukelekian and Heller (*69*), by courtesy of the American Society for Microbiology.

Braafladt (*47*) reported that bacterial growth was reduced in the presence of kaolin. A reduction in the growth rate of *A. chroococcum* and *E. coli* in the presence of calcium phosphate gel (*97,117*) was attributed to sorption of the gel on the bacterial surface and a subsequent reduction in the rate of diffusion of substrates to the cell surface.

C. Microbial Ecology

As compared with simple aqueous systems, selective sorption of certain microorganisms on soil particles (*6,7,11*) is one of the most important factors whereby the solid phase of the soil exerts some control over the balance between populations of microorganisms in soils. Because of differences in the sorptive capacities of various soils resulting from variations in the size, exchange capacity, and types of particles present, the composition of the microbial populations in soils also must vary markedly. The concentration of nutrients at the surface of particles must favor the readily sorbed microorganisms (*69*), while the sorption of inhibitory substances such as antibiotics (*118–123*) might have a selectively detrimental effect on sorbed cells. This selective sorption of microorganisms by soil colloids has been employed by Krishnamurti and Soman (*30*) to separate mixtures of different species of bacteria.

Little emphasis has been placed on the importance of sorptive interactions between microorganisms and soil particles in studies of the microbial ecology in soils. Outstanding examples of the importance of such interactions are the correlations between the rapid spread of *Fusarium oxysporum* f. *cubense* (*124*) and *Histoplasma capsulatum* (*125*) in certain soils and the lack of montmorillonite-type clays in such soils. The so-called "long-life" soils of Central America, in which *F. oxysporum* f. *cubense* fails to spread, all contain montmorillonite. Detailed studies of the effects of various clay minerals on the metabolism of *Fusarium* and typical soil bacteria have led Stotzky (*85–88*) to suggest that montmorillonite regulates the pH of the microbial environment. Changes in pH induced by bacteria may limit the growth of these acid sensitive bacteria in the absence of montmorillonite, while growth of the fungus is unaffected by the lower pH. Montmorillonite probably stimulates bacterial development by maintaining the pH at levels favorable for the bacteria and probably encourages competitive exclusion of *F. oxysporum* f. *cubense* (*126*). This work has been summarized recently by Stotzky (*127,128*). Skinner (*128a*) has described a situation in which the growth of *Fusarium culmorum* was suppressed by *Streptomyces albidoflavus* even when the antibiotic produced by the streptomycete was inactivated by the addition of bentonite to sand cultures. He attributed this effect to a competitive interaction between the two organisms.

Several authors have suggested that soil texture is an important factor in the survival of root nodule bacteria in soils exposed to desiccation and high temperatures (*129–132*). The success in overcoming survival problems in a field situation by the addition of montmorillonite to a sandy soil (*133*) prompted an investigation by Marshall (*134*) of the effects of colloidal materials on the susceptibility of *Rhizobium* species to desiccation and high-temperature treatment. Fast-growing strains of *Rhizobium* failed to survive in dry gray and yellow sandy soils heated to 70°, while excellent survival was found in red sands and soils of heavier texture. Addition of montmorillonite or illite to a gray sandy soil protected *R. trifolii* from these extreme conditions. Kaolinite, on the other hand, failed to protect this organism (Table 14-10).

TABLE 14-10

Survival of *R. trifolii* TA1 in a Gray Sand and in Sand Amended with 5% of Various Clay Minerals (*134*)

Soil treatment	Initial inoculum, $\log_{10}$ viable cells/g soil	Heat treatment after drying soil at 30°, $\log_{10}$ viable cells/g soil		
		Nil	50°	70°
Sand	8.55	6.27	4.58	<1.00
Sand plus kaolinite	8.55	5.59	4.19	<1.00
Sand plus montmorillonite	8.55	6.58	6.36	5.50
Sand plus illite	8.55	6.43	6.04	5.31

It was proposed that the illite and montmorillonite might influence the survival of the cells by forming a protective envelope around each cell, thereby modifying the rates of water movement into and out of the cells during drying and rewetting (*23,33*). Slow-growing *Rhizobium* species appeared to be remarkably resistant to dry heat in the absence of clays (*134*). It has been suggested (*73*) that the widespread distribution in arid and semiarid regions of legumes associated with slow-growing rhizobia may result from the ability of these organisms to survive exposure to high temperatures in dry, sandy soils. The limited distribution of fast-growing bacteria in such areas may be related to their low resistance to heating and the need for the protective effects of certain soil colloids.

Other protective phenomena that probably involve sorptive interactions between microorganisms and solid particles are the effect of peat on *Rhizobium* exposed to high temperatures (*135*), kaolin on bacteria exposed to desiccation (*136*), and montmorillonite on *Azotobacter* subjected to x-irradiation (*137*). Miyamoto (*138*) has found that soil-borne viruses survive

for comparatively long periods when sorbed to clay particles. Sorption of bacteriophages on clay particles (*139*) may protect phage-susceptible bacteria from infection.

D. Soil-Forming Processes

In view of the occurrence of sorptive interactions between microorganisms and soil particles, the decomposition of aluminosilicates by bacteria (*140,141*) is probably an important factor in soil development. A number of Soviet microbiologists have claimed that microorganisms are essential in the process of soil formation from rock materials (*142,143*). They claim that microorganisms, especially lichens, sorbed to rock surfaces result in the dissolution of rock components and that following decomposition of the microbial cells a type of "clay-humus" residue remains. The microbial metabolites responsible for rock decomposition generally function by chelation mechanisms. The metabolites include lichenic acids (*144*), 2-ketogluconic acid (*145*), and various organic acids (*146*).

Khudyakov, quoted by Kononova (*147*), has noted that the accumulation of humus in soils is directly related to the exchange capacity of soil minerals. This phenomenon was attributed to the sorption of bacteria by the colloids of such soils, resulting in a decrease in their metabolic activity and the subsequent accumulation of organic materials. Kobus and Pacewiczowa (*148*) recently reported that the addition of 20% clay to a sandy soil markedly reduced the rate of organic matter breakdown. Similarly, Filip (*149*) found a decrease in the mineralization of organic materials and an increase in the accumulation of humic substances when increasing amounts of bentonite were added to sand cultures.

Undoubtedly, sorbed microorganisms are also important in mineral transformations, aggregate formation, and in other important soil processes. These aspects of sorptive interactions between microorganisms and soil particles deserve more detailed study in the future.

14-7. CONCLUDING REMARKS

It is obvious that a good deal of confusion exists as to the effects of sorptive interactions on the behavior of microorganisms in soils, and further investigations are required to fully understand the mechanisms involved in these interactions. Our present state of knowledge is such that it can be stated quite emphatically that microbial behavior in soils is modified tremendously as a result of sorptive interactions between microorganisms and the solid phase of soils. Consequently, a plea is made for more realistic studies of microbial activity in natural soils. Such investigations must take account of

the selectivity of sorptive processes, the surface properties of the microorganisms and of the soil particles, the cation status of the soils, and the organization of the solid constituents of the soils. Critical studies of these sorptive interactions are essential if we are to obtain a meaningful insight into the activities of microorganisms in complex natural environments such as soils.

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